Effect of Adrenaline, Noradrenaline and Insulin on Mg^{++} Dependent ATPase

In the present paper the authors wish to establish the effect of some biologically important materials on the activity of Mg^{++} dependent adenosine triphosphatase (ATPase) in the erythrocyte membrane. Because the connection between the activity of Mg^{++} dependent ATPase and the passive transport of Na^+ ions is considered in some papers[1–3], the hormones adrenaline, noradrenaline and insulin were used which may have a certain relation to passive permeability of ions. Adrenaline changes the fluxes of Na^+ and Cl^- ions[4] and noradrenaline the fluxes of K^+ ions[5,6]. The relation between insulin and the permeability of Na^+ and K^+ ions was mentioned by ZIERLER[7] and in several papers quoted by SCHOFFENIELS[8].

Materials and methods. In the experiments, to 0.1 ml of packed fresh human erythrocytes 1 ml of aqueous hormone solutions were added. After 6 min treatment at room temperature the erythrocytes were incubated in the medium which included hormone, 1.33 m*M* ATP, 2.65 m*M* $MgCl_2$ and 0.2 *M* *Tris*-HCl buffer (pH = 7.4) at 37°C for 1 h. The final incubation volume was 3 ml. The hormones used were products of the firm Spofa: injection of 'Adrenalinum hydrochloricum solutum 1:1000', injection of '1-Noradrenalinum hydrotartaricum in solutione aquosa sterili 1:1000', injection of 'Insulin' contained 40 IU in 1 ml. The activity of Mg^{++} dependent ATPase was determined from the amount of phosphorus split off during incubation. The amount of phosphorus was estimated according to FISKE and SUBBAROW[9]. The activity of Mg^{++} dependent ATPase was expressed by the μmoles of P split off by 0.1 ml of erythrocytes during one hour's incubation under the given conditions.

Results and discussion. In all experiments, adrenaline and noradrenaline enhanced the activity of Mg^{++} dependent ATPase. There were, however, some individual differences in the extent of their effect, if erythrocytes from different persons were used. In 9 experiments performed, some of the erythrocytes were more sensitive to adrenaline, whereas others to noradrenaline. 2 examples of various adrenaline and noradrenaline action are given in Figure 1. The reason for this different response of the blood cells to both hormones has not been investigated.

An addition of more than 10 IU of insulin to 0.1 ml of erythrocytes stimulated the activity of Mg^{++} dependent ATPase (Figure 2). There were small differences in the effect of insulin on the erythrocytes of different healthy donors. The stimulation of Mg^{++} dependent ATPase activity caused by adrenaline, noradrenaline or insulin was not changed in the presence of 5×10^{-4} *M* ouabain which inhibits the Mg^{++}, Na^+, K^+ dependent ATPase.

The hormones enhance the activity of Mg^{++} dependent ATPase only in high concentrations; however, exogenous ATP is not cleaved by intact erythrocytes under physiological conditions at all[10,11], so that eventual alterations in the membrane caused by lower adrenaline, noradrenaline or insulin concentrations cannot be established by the present techniques.

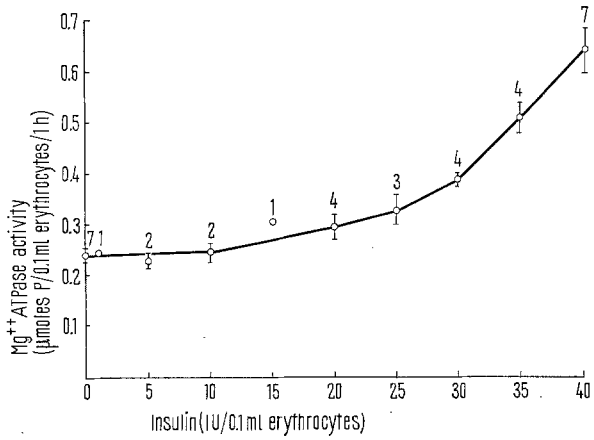

Fig. 2. Influence of insulin on the activity of Mg^{++} dependent ATPase. Results are expressed as mean ± S.E.M. Figures refer to the number of experiments.

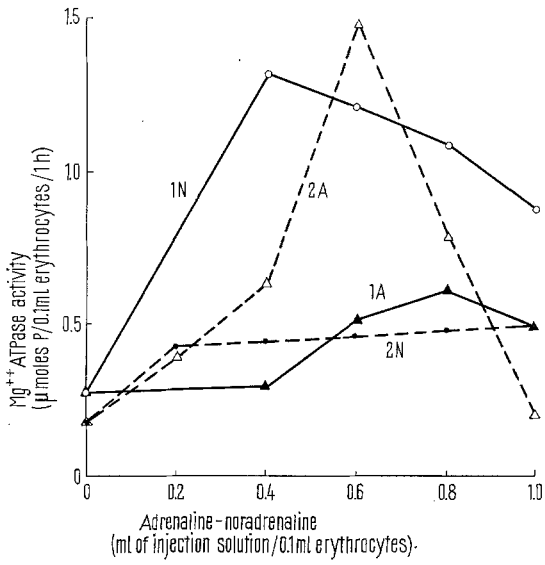

Fig. 1. Influence of adrenaline (A) and noradrenaline (N) on the activity of Mg^{++} dependent ATPase in erythrocytes of 2 blood donors (1) and (2). The highest amount of adrenaline in the incubation medium corresponds to the concentration 1.8–2 m*M* of noradrenaline.

The enhanced activity of Mg^{++} dependent ATPase after adrenaline, noradrenaline or insulin treatment can be explained either by the direct activation of the enzyme by hormones or by a secondary conformation change in the molecule of the enzyme, due to primary change in other component(s) of the membrane. We cannot exclude the possibility either that the hormones do not influence

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the activity of the enzyme but only enhance the permeability of ATP through the membrane to the active enzyme centre.

The activity of Mg^{++} dependent ATPase in the erythrocyte membrane is enhanced in addition to insulin by adrenaline or noradrenaline which influence the contraction of smooth muscle. Does this fact indicate a certain relation of Mg^{++} dependent ATPase to contractile ghost protein[12]? A working hypothesis is considered that Mg^{++} dependent ATPase could form a part of the region of the pores, by which some substances penetrate through the membrane.

Zusammenfassung. Die Hormone Adrenalin oder Noradrenalin stimulieren in menschlichen Erythrozyten die Aktivität der Mg^{++}-abhängigen ATPase. Die Aktivität dieses Enzyms ist auch nach der Wirkung des Insulins gesteigert.

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Protein Synthesis in Isolated, Beating Rat Atria

To study further the pathogenesis of cardiac hypertrophy[1–4], and to increase understanding of the control of cardiac protein synthesis, it would be useful to be able to study the latter process in vitro. The spontaneously beating rat atrium lends itself to such studies, particularly to investigation of interventions affecting cardiac protein synthesis over short time periods.

Methods. Male, Sprague-Dawley rats were anesthetized with ether and their hearts quickly removed. Both atria were separated from the ventricles and placed into 25 ml Erlenmeyer flasks. This procedure required less than 2 min. 5 ml of Tyrode's medium containing glucose (5 µm/ml) and L-leucine (1.0 µm/ml) served as the incubation medium. L-leucine-1-^{14}C was added to yield a final specific activity of 0.25 µc/µm. The effect on incorporation rates of variation in leucine concentration was examined by also incubating atria with 0.25, 0.50, or 4.0 µm/ml leucine, with the specific radioactivity held constant. The medium was equilibrated before use with 95% O_2/5% CO_2 to yield a pH of 7.4 at 37°C, 7.2 at 30°C and 7.0 at 22°C. At the end of incubation the atria were homogenized and total atrial protein was prepared for radioassay as described elsewhere[5].

The rate of leucine uptake from the medium was determined as follows: after preincubation for 1 h in non-isotopic medium, the atria were transferred to medium containing leucine-1-^{14}C and allowed to incubate for either 10 or 30 min. The tissue was then rinsed quickly 3 times, blotted and homogenized in exactly 3.0 ml of 5% TCA. Protein was precipitated for at least 3 h at 0–4°C. A 0.5 ml aliquot of the supernate was radioassayed in a liquid scintillation system. Paper chromatography confirmed that all counts were in ^{14}C-leucine. The increment in activity in the 30 min over the 10 min sample confirmed that uptake of leucine had occurred. Further 15 min uptake experiments then were used for the data reported herein.

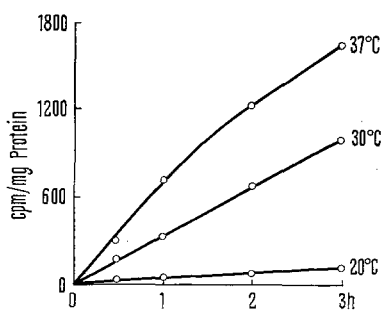

Fig. 1. The dependence of total atrial protein synthesis upon incubation time and temperature. Note that at each temperature protein synthesis is linear for at least 2 h. In the 37 °C system, there may be some fall in rate by the 3rd h of incubation.

Results. The linear incorporation of leucine into atrial protein as a function of time at various temperatures is shown in Figure 1. The relatively high rate of isotope incorporation into atrial protein at 30°C, compared with that in rat ventricular, kidney and liver slices and in whole rate diaphragm, is shown in Table I. The differences do not seem due solely to differences in leucine uptake (Table II). The effect on isotope incorporation of varying concentrations of leucine in the medium is shown in Figure 2. Above 0.5 µm/ml the final specific activity of total atrial protein is maximal. Progressive increments in leucine uptake occur at each comparable concentration (Figure 3). Protein synthesis is about 25%

Table I. Incorporation of leucine-1-^{14}C into total protein by various rat tissue preparations

Tissue	Specific activity (cpm/mg protein)
Ventricular slices	76 ± 10[a]
Intact diaphragm	116 ± 12
Kidney slices	278 ± 23
Liver slices	423 ± 18
Intact, beating atria	623 ± 50

[a] Mean ± S.E. of the mean.

Table II. Uptake of leucine-1-^{14}C from the incubation medium by various rat tissue preparations

Tissue	Uptake (µm × 10^3 leucine/100 mg wet wt.)
Kidney slices	57 ± 1.5[a]
Liver slices	45 ± 1.5
Intact, beating atria	51 ± 1.5

[a] Mean ± S.E. of the mean.

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